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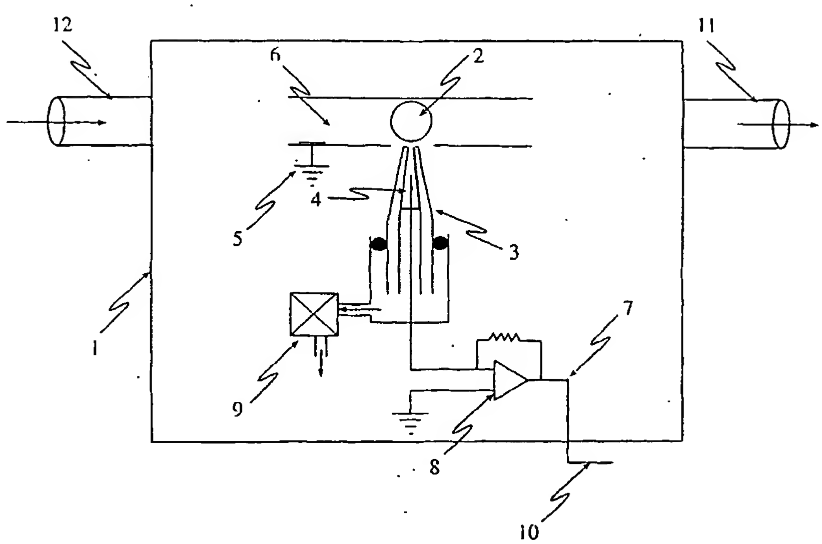
(54) **Title:** SINGLE CELL AUTO PATCH



(57) **Abstract:** The invention provides a method for obtaining a patch clamp recording from a cell, which includes the steps of providing a microchannel capable of an axial flow of a liquid, providing in the microchannel at least one access port to allow radial access from the exterior of the microchannel (air) to the interior of the microchannel (liquid); whereby liquid in the microchannel forms a meniscus at the port and produces an air/liquid interface, providing a patch-clamp pipette having a tip suitable for passing into the access port suitable for forming a high-resistance (giga-ohm) electrical seal between the tip and the cell, passing liquid carrying the cell axially along the microchannel, causing the cell to be carried to the access port, moving the patch-clamp pipette tip and the microchannel relative to each other radially to bring the tip into contact with the air/liquid interface in the access port, applying suction to the patch-clamp to draw the cell onto the tip to form the seal, and making a patch-clamp recording. The invention

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

SINGLE CELL AUTO PATCH

The system is based on delivery of cells via a system of microchannels to a patch clamp pipette. Cells may be pre-sorted using a fluorescence activated cell-sorter (FACS) or other methods of sorting such as immunomagnetic selection. However the system could also be used without pre-sorting for homogenous cell populations. Delivery of cells and events leading to and including a patch clamp recording are computer controlled as is subsequent drug delivery.

A patch-pipette accesses cells as they pass an access port in the microchannel. High-resistance electrical seals between pipette and cell (in the order of G or more) are achieved by applying suction to the pipette via a suction controller either on a continuous basis or triggered by the FACS detector which also diverts cells with an appropriate fluorescence signal or light scattering properties along the appropriate microchannel. The minimal system would consist of a single microchannel with patch-clamp module. Cell suspensions are pumped (eg. using a peristaltic pump) from a cell incubator through the microchannel. More sophisticated and also higher throughput devices would have a FACS with multi-wavelength capability to permit selection of several cell-types and also multiple patch-clamp modules to permit parallel recording from many cells which may be different or the same in respect to their fluorescence or cell-scattering 'signature'.

The process in essence consists of:-

1) Cells scanned by FACS (or not as the case may be) and a cell or cells having appropriate fluorescence signal diverted along microchannel toward a patch-clamp module.
2) Suction is applied to the patch-pipette located in the patch clamp module either at the same time or according to some fixed predetermined interval such that

suction occurs as the selected cell passes an access port in the microchannel whereby the cell is drawn to the pipette tip.

3) Seal resistance is monitored automatically and suction controlled by feedback mechanism under control of a computer. Subsequent steps involved in standard patch-clamping are also determined under software control.
4) Once the desired patch-clamp configuration has been achieved, a perfusion flow controller switches the flow of solution through the microchannel from delivering cells to delivering drug solutions and the experiment is initiated.

Patch-clamp modules may be cascaded such that in the event more than one cell is detected by the FACS, multiple recordings may be made. Excess cells are simply recycled back to the cell incubator.

In the case of a homogeneous source of cells, the FACS front end is not required although it would have the beneficial effect of eliminating debris from the system. In the case where cells come from a mixed background a FACS front end allows selection of even minor components of the overall cell suspension.

The system can be fully automated and because it also recycles cells and solutions, can run for extended periods of time without intervention. A device for supplying conventional glass patch pipettes will be incorporated or alternatively a system for rejuvenating and hence re-using quartz glass pipettes will be used.

Data obtained can be automatically downloaded to a server for off-line analysis etc. without interrupting data acquisition.

Legends

Figure 1. Flow Patch-Clamp System

1. fluorescence-activated cell sorter (FACS)- optional
2. cell incubator
3. patch-clamp module
4. control and data acquisition interface
5. perfusion flow controller
6. sorted cells: channel #1
7. sorted cells: channel #2
8. unsorted cells channel
9. return channel to cell incubator
10. waste
11. computer workstation to control system

Figure 2. Patch-Clamp Module

1. Patch-clamp module
2. Cell
3. Patch-clamp pipette
4. Pipette filling solution (electrolyte)
5. Earth connection
6. Bathing solution
7. Patch-clamp output (membrane current)
8. Patch-clamp amplifier
9. Suction control system
10. Control and data acquisition line
11. Microchannel outflow
12. Microchannel inflow

Figure 3. Perfusion Flow Controller

1. Perfusion flow controller
2. Inflow from FACS
3. Inflow from drug application system
4. Manifold and controller to switch between drug solutions
5. Multiwell plate containing drug solutions

<u>Claims</u>

1. A method for obtaining a patch clamp recording from a cell, which includes the steps of:

(i) providing a microchannel capable of carrying an axial flow of a liquid;
(ii) providing in the microchannel at least one access port to allow radial access from the exterior of the microchannel (air) to the interior of the microchannel (liquid); whereby liquid in the microchannel forms a meniscus at the port and produces an air/liquid interface at the port;
(iii) providing a patch-clamp pipette having a pipette tip suitable for passing into the access port suitable for forming a high-resistance (giga-ohm) electrical seal between the tip and the cell;
(iv) *passing liquid carrying the cell axially along the microchannel, causing the* cell to be carried to the access port;
(v) moving the patch-clamp pipette tip and the microchannel relative to each other radially to bring the tip into contact with the air/liquid interface in the access port;
(vi) applying suction to the patch-clamp pipette to draw the cell onto the tip to form the seal; and
(vii) making a patch-clamp recording.

2. A method according to claim 1 in which the cell has been sorted or selected from a heterogeneous source of cells.

3. A method according to claim 2 in which the cell has been sorted and selected using a Fluorescence Activated Cell-Sorter (FACS).

4. A method according to any preceding claim in which a plurality of cells are carried to the access port singly in a sequential flow.

5. Apparatus for carrying out the method of any of claims 1 to 4, comprising:

(i) a microchannel capable of carrying an axial flow of a liquid; the microchannel having an access port to allow radial access from the exterior of the microchannel to the interior; and

(ii) a patch-clamp pipette having a pipette tip suitable for passing into the access port.

6. Apparatus according to claim 5 where the cross-sectional microchannel dimension permits only one cell to pass the access port at a time.

7. Apparatus according to claim 5 or 6 wherein the microchannel is tubular.

8. Apparatus according to claim 7 wherein the diameter of the tubular microchannel is between 1 and 2 times the diameter of a cell.

9. Apparatus according to any of claims 5 to 8 wherein the microchannel has more than one access port spaced axially.

10. Apparatus according to any of claims 5 to 9 wherein there is more than one microchannel.

Figure 1

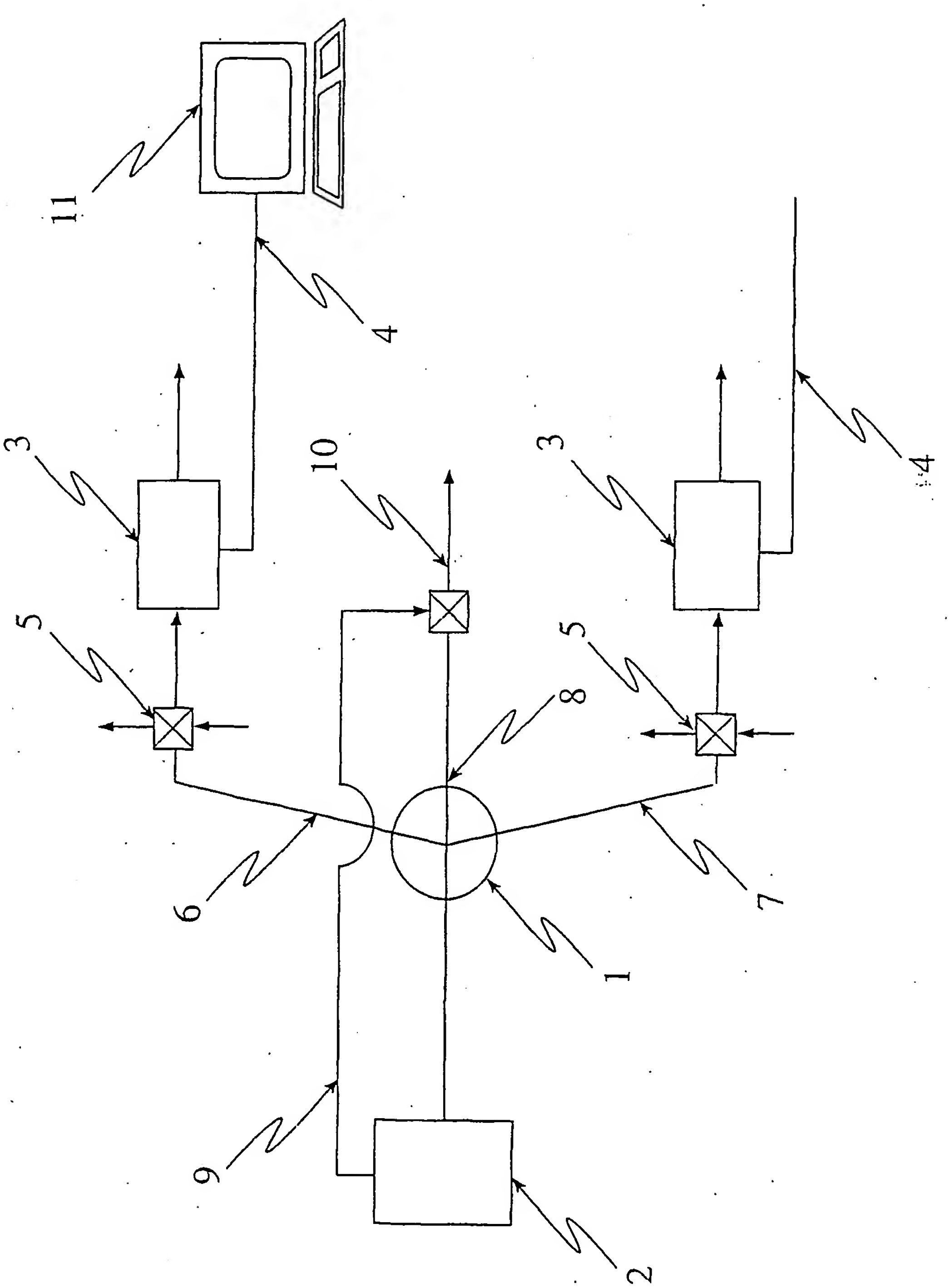

Figure 1

Figure 2

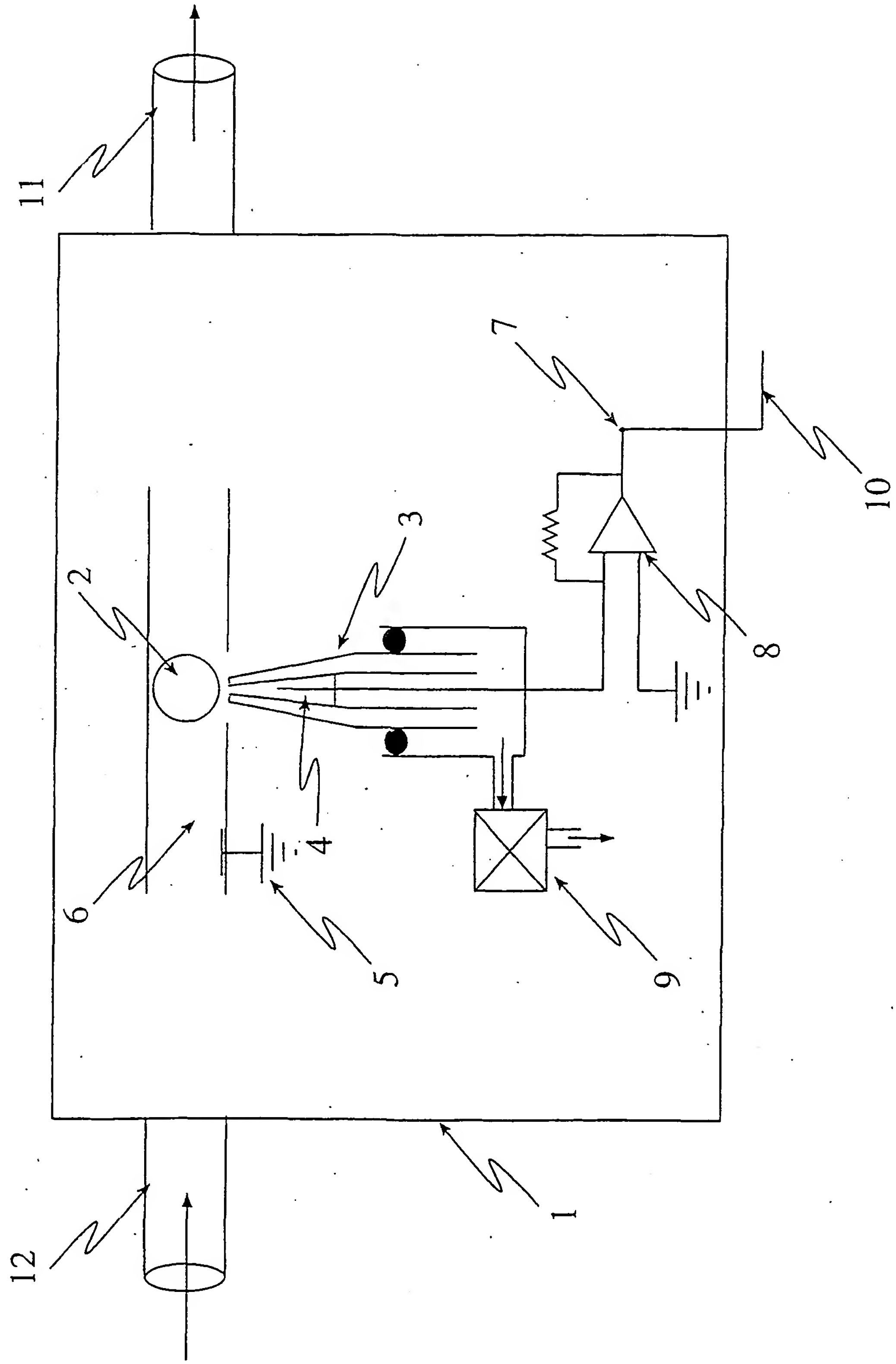

Figure 2

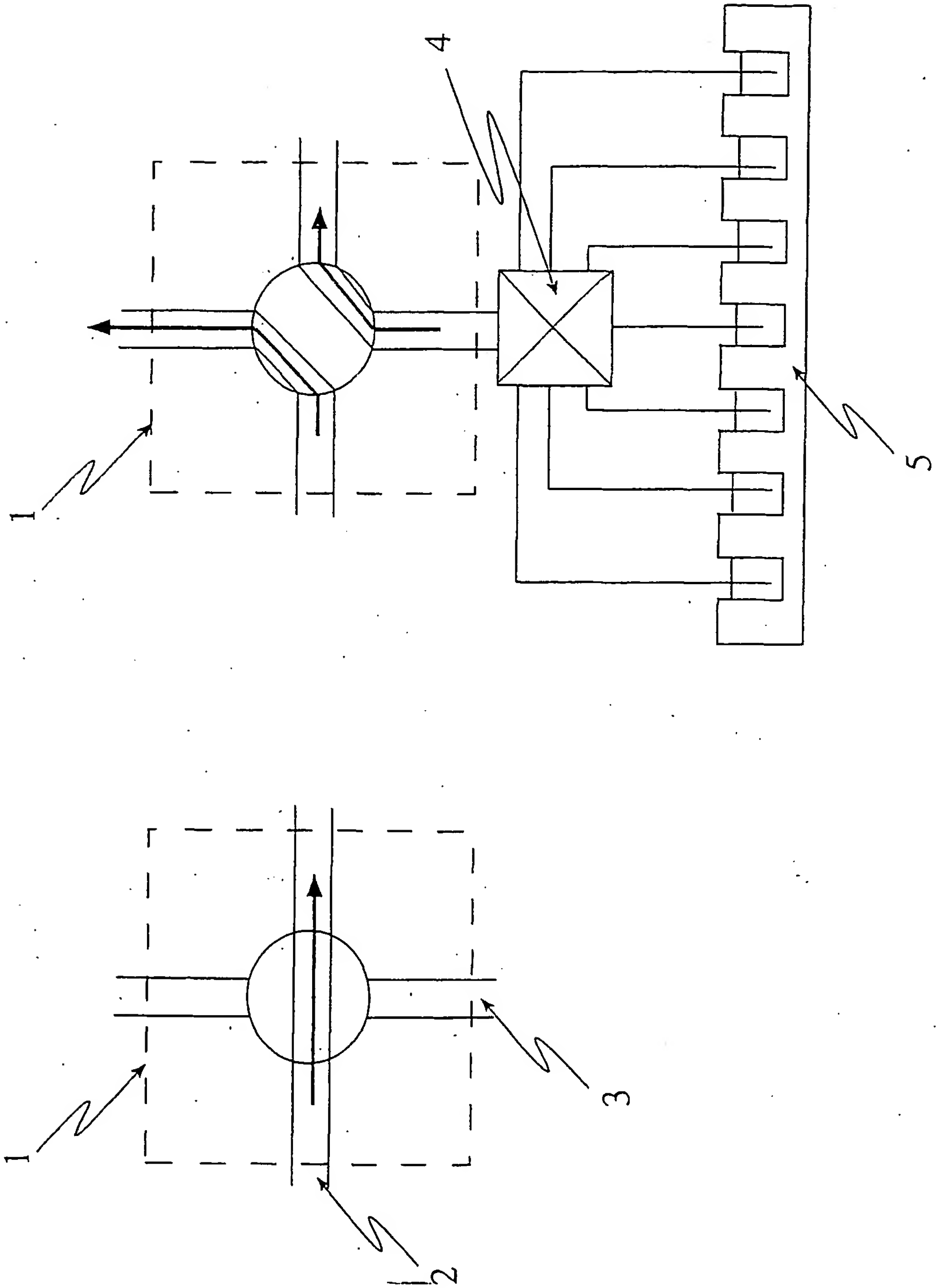

Figure 3

Figure 3

INTERNATIONAL SEARCH REPORT

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PCT/GB 01/02490

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/487 C12M1/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12M F16L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	US 6 063 260 A (OLESEN SOEREN-PETER ET AL) 16 May 2000 (2000-05-16) the whole document ---	1-10
A	HAMILL O P ET AL: "IMPROVED PATCH-CLAMP TECHNIQUES FOR HIGH-RESOLUTION CURRENT RECORDING FROM CELLS AND CELL-FREE MEMBRANE PATCHES" PFLUEGERS ARCHIV, SPRINGER VERLAG, BERLIN, DE, vol. 391, 1981, pages 85-100, XP000196663 ISSN: 0031-6768 the whole document ---	1-10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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